accompanying table are as follows: *S. Chelsoni* = *S.* × *chelsonii* (i.e. *S. rubra* × *purpurea*), *S. Drummondii* = *S. leucophylla*, *S. Maddisoniana* = *S.* × *formosa* (i.e. *S. psittacina* × *minor*), *S. Moorei* = *S.* × *moorei* (i.e. *S. leucophylla* × *flava*), *S. Popei* = *S.* × *popei* (i.e. *S. rubra* × *flava*), *S. rubra acuminata* = *S. rubra* subsp. *rubra*, *S. Stevensii* = *S.* × *catesbaei* (i.e. *S. flava* × *purpurea*), *S. Williamsii* = *S.* × *catesbaei* (i.e. *S. flava* × *purpurea*). The references to "*S. patersoni*" in the list written *ca.* 1900 present interesting problems since apparently such crosses did not exist in or before 1884. (Note #7 supplied by the editors.)

Cultivation of *Triphyophyllum peltatum* (Dioncophyllaceae), the Part-Time Carnivorous Plant

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Summary

Cultivation of *Triphyophyllum peltatum*, a rarely grown part-time carnivorous plant, is presented.

Introduction

Comparatively little is published on *Triphyophyllum peltatum* (Hutch. & Dalz.) Airy Shaw, a liana widely neglected among carnivorous plant students and growers. Almost nothing has been published on the cultivation of this plant. In the course of our investigations of the secondary metabolites of the plant order Nepenthales, especially of the naphthylisoquinoline alkaloids (Bringmann & Pokorny 1995[1]) that are so far known only from two small palaeotropical families, *viz.* Ancistrocladaceae and Dioncophyllaceae, we became interested in the physiology and biochemistry of these plants. Live material is indispensable for the study of the biosynthesis of the plant natural products. Therefore, efforts were made to obtain living specimens of several species of *Ancistrocladus* (the only genus of Ancistrocladaceae) and of *Triphyophyllum peltatum* (one of three monotypic genera of Dioncophyllaceae, and the only carnivorous plant to contain naphthylisoquinolines, Bringmann *et al.* 1998a). We succeeded in finding seeds and small plants of a number of these species, including *T. peltatum*. Our experience with germination and cultivation, including *in vitro* methods, are communicated in this paper.

[1]Part 124 in the series "Acetogenic Isoquinoline Alkaloids". For part 123, see Bringmann *et al.* 1998c.

General

T. peltatum is a high forest liana that grows in coastal rain forests of tropical West Africa (Airy Shaw 1952). *Triphyophyllum* means "three kinds of leaves," which characterizes this particular genus. During most of its life cycle it is not a carnivorous plant. In its juvenile phase it forms rosettes of undivided lanceolate leaves with obtuse to acute leaf tips. Once the young plant attains a certain age and height (ca. 25-40 cm), on the onset of the next rainy season, carnivorous leaves are formed that have a more or less reduced lamina and a prolonged midrib beset with glandular emergences (Figure 5, p9). These glands secrete a sticky mucilage (Marburger 1979) and capture invertebrates (Green *et al.* 1979). The uptake of the amino acid L-alanine by the carnivorous leaves of *T. peltatum* has been demonstrated recently (Bringmann *et al.* 1998b). Later on, the plant climbs rapidly into the canopy of high trees, attaching to supporting plants by leaves that have two hooks curling towards the leaf base at either side of the leaf tip (Dioncophyllaceae means "plant family with double-hooked leaves"). While the white or pale pink flowers are not spectacular, the seeds are unique within the plant kingdom. Unlike most angiosperm fruits, those of Dioncophyllaceae open prior to seed maturity. These plants have, therefore, been called "secondary gymnosperms" although they are far from being related to the conifers. The large (5-12 cm across), disk-shaped seeds are borne on prolonged, thickened and even woody funiculi (seed stalks) attached to the centre of the disks. The embryo is located close to the point of attachment in a thickened ring of white endosperm approximately 1 cm in diameter (Figure 1), the rest of the seed consists of a thin, papery wing that is slightly striated radially (Figure 2). The detached seeds are able to fly for considerable distances until they reach the floor of the rainforest.

Figure 1: Cross-section of ripe seed of *T. peltatum* with removed wing. (Photo: H. Rischer)

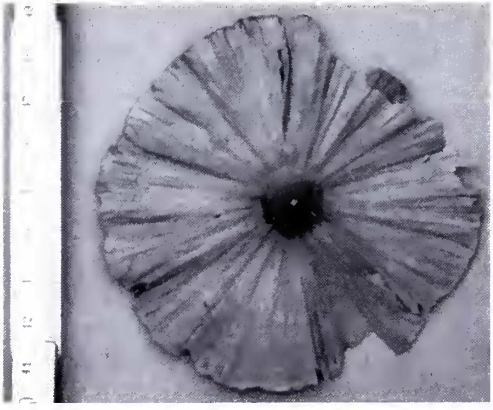

Figure 2: Ripe seed of *T. peltatum*. (Photo: H. Rischer)

Habitat

Being a West African endemic, *T. peltatum* grows in the Ivorio-Liberian block of the Western forest massif. It is quite frequently found in the Taï National Park, which belongs to the Guineo-Congolese domain. The dense humid evergreen forest is classified as a "*Eremospatha macrocarpa* and *Diospyros mannii* forest," a fundamental type determined by climatic and soil conditions (Guillaumet & Adjanohoun 1971). The component tree species (like *Eremospatha macrocarpa* (Mann &

Figure 3: Seedling of *T. peltatum* just after germination. (Photo: H. Bringmann)

Figure 4: Same plant as in Figure 3 a few days later with shed seed coat. (Photo: H. Bringmann)

Figure 5: Carnivorous specimen of *T. peltatum* at Centre National de Floristique, Abidjan, Ivory Coast. (Photo: H. Bringmann)

Wendl.) Wendl., *Diospyros mannii* Hiern, *Diospyros gabunensis* Gürke, *Maranthes chrysophylla* (Oliv.) Prance, *Chrysophyllum perpulchrum* Mildbr. ex Hutch. & Dalz., *Chidlowia sanguinea* Hoyle, etc.) are found in other communities, but in lower abundances. The lowland community is a "*Diospyros spp.* and *Mapania spp.* forest," as typified by *Mapania* (Cyperaceae) and *Tarrietia utilis* (Sprague) Sprague (Sterculiaceae) growing in clay soils.

Above all, the vegetation of the South West of Ivory Coast is marked by the specific endemism of the flora containing particular taxa called "Sassandrians," a term used to describe the species that occur in the shady forests in the West of Ivory Coast between the rivers Sassandra and Cavally. Independent from the presence of *T. peltatum*, this particular flora contains the following species: *Androsiphonia adenostegia* Stapf (Passifloraceae), *Cassipourea hiotou* Aubrév. & Pellegr. (Rhizophoraceae), *Cola buntingii* Bak. f. (Sterculiaceae), *Crinum scillifolium* A. Chev. (Amaryllidaceae), *Gilbertiodendron robynsianum* Aubrév. & Pellegr. (Caesalpiniaceae), *Guarea leonensis* Hutch. & Dalz. (Meliaceae), *Hypolytrum schnellianum* Lorougnon (Cyperaceae), *Ouratea amplectens* (Stapf) Engl. (Ochnaceae), *Sciaphila africana* A. Chev. (Triuridaceae), *Soyauxia grandifolia* Gilg & Stapf (Medusandraceae), *Thomandersia anachoreta* Heine (Acanthaceae), etc. In Sierra Leone and Liberia, *T. peltatum* exists under the same ecological conditions.

Cultivation of *T. peltatum* in the Greenhouse

We received uprooted, juvenile but post-carnivorous *T. peltatum* specimens. In Würzburg, these were planted in fine grain, #2 Lecaton® (from Leca, Pinneberg, Germany) substrate in pots 10 cm wide and 20 cm tall. This substrate does not rot under greenhouse conditions. The high pots provide enough depth for the roots to descend a considerable distance into the substrate. Although they suffered from desiccation when the greenhouse door was left open, they grew well for a few years, and produced long shoots with hooked leaves, some of which were several meters long with short shoots that bore normal leaves sprouting from the axils of the hooked leaves. Eventually, however, they all died. Examination of the roots showed that no growth below ground had ever taken place after the plants were uprooted and transported. Similar plants grown in shaded greenhouses in Abidjan showed new root growth after 3 to 6 months, with an average survival rate of 40 to 50%. Especially when the plants are grown in the temperate zone, the sensitive root system should not be disturbed once the plants have been established. This means that there are only two ways to obtain normally growing plants, *viz.* by seed or by growing rooted plants without removal of the substrate.

Seed is extremely difficult to obtain because it is set during the rainy season, when traveling to the localities of *T. peltatum* is very troublesome. Furthermore, to harvest seed one must climb high trees since seeds detached by the wind will fly and disappear into the dense ground vegetation. Thus, it took more than ten years before one of us (L.A.A.) finally reached fruiting plants in May 1997. The seeds were sown immediately in Abidjan and in Würzburg under greenhouse conditions. In Abidjan the seeds were planted *ca.* 3 cm deep in sandy potting soil and watered frequently. The germination rate was ca. 80%. In Würzburg, flat dishes were used with a 2 cm layer of Lecaton®, which was kept permanently wet by a constant water level of 1 cm below the surface of the substrate (maintained by a drainage hole at one side of the dish). The wings of the seeds were removed in order to prevent excessive fungal/algal proliferation, and the seeds were sown on the surface of the substrate. The dishes were kept in a light place without direct sunlight at 22°C and almost 100% humidity. Germination (*ca.* 50%) took place after a few weeks to several months. The cotyledon tips remained in the seed coat in intimate contact with

the endosperm (cryptocotylar germination). The hypocotyl and root pushed their way out of the seed at the former point of attachment of the funiculus. These observations correspond to those by Schmid (1964) with *Habropetalum dawei*, the only other member of Dioncophyllaceae hitherto reported to have been germinated and grown for several months outside Africa. The growing hypocotyl then lifted the seed up and the cotyledons bent sidewards at their base. The primary leaves sprouted from a narrow slit between the cotyledons where they were attached to the hypocotyl (Figures 3 and 4). These were the first rosette leaves. The rosettes produced leaf after leaf and grew in length without an obvious correlation to the changing (outdoor) seasons of temperate climate. Light intensity was kept constant by artificial lighting (between 8 h and 20 h) so that a mean light sum of 340 klx h was reached daily. Some of the rosette leaves reached a length of more than 30 cm. A one-year old plant with 20 cm long leaves was displayed at the Second Conference of the ICPS in Bonn at the end of May 1998.

In addition, rooted plants of *T. peltatum* in their substrate were sent to Würzburg in summer 1997. These plants recovered remarkably well from the transport and are still alive. Both the germinated specimens and the plants sent with roots are now being acclimatized to normal warmhouse conditions (average 22°C, occasional minimum of 17°C at about 7 a.m. maximum of 28°C at 6 p.m., average 83% humidity, occasional minimum 53%, maximum 95%, *cf.* typical plots on Figure 6) because excessive humidity led to algal growth on the leaves and favoured other pests, especially aphids and scale. Larvae of fungus gnats, which are favoured by the required high temperatures and intense watering and which cause severe damage especially on the sensitive roots of young plants, are controlled by continuous treatment with predator nematodes (Nemalogic®, from Sautter & Stepper, Ammerbuch, Germany). The plants are still growing (see Front Cover), and some have attained a size that corresponds to carnivorous specimens in the wild. We

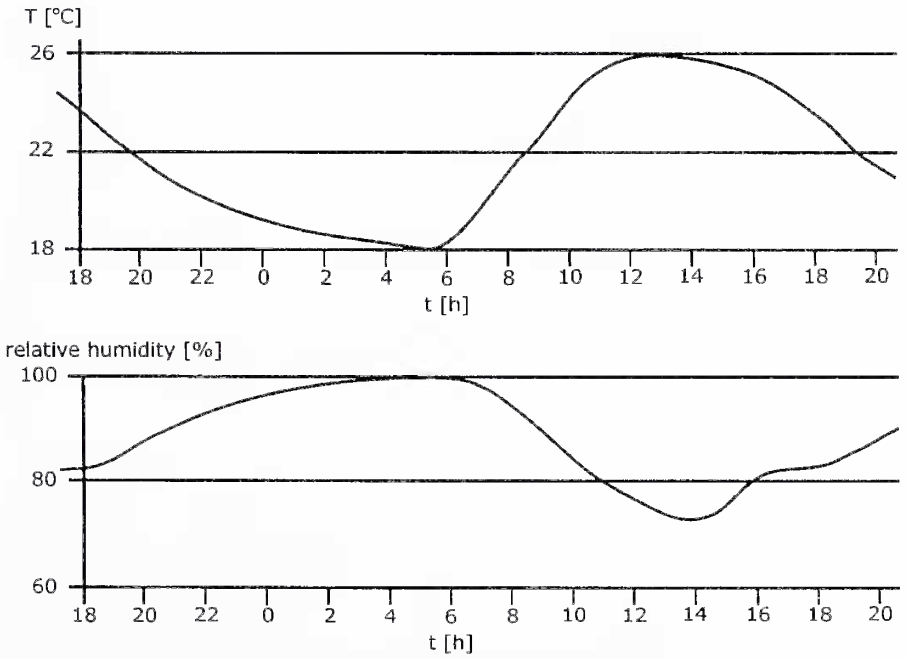

Figure 6: Typical plots of temperature and relative humidity for the greenhouse cultivation of *T. peltatum* at the Botanical Garden of Würzburg.

have, however, not yet obtained carnivorous leaves. Less than 5% of the seedlings have died. This is the first successful germination of seeds of *T. peltatum* achieved in cultivation.

in vitro Cultivation of *T. peltatum*

Axenic *in vitro* cultivation of plant material facilitates both the propagation of the plants and the investigation of their physiology. There are apparently no reports in the literature on the tissue culture of *T. peltatum* or any other member of Dioncophyllaceae. We sowed some of our seeds in sterile conditions because greenhouse-grown plant material is difficult to surface-sterilize. The lack of experience with the sterilization of these seeds and the unique occasion of having viable material encouraged us to try several simultaneous approaches. The wings were removed, and the remaining centres were surface-sterilized using 70% ethanol for ten seconds and a sodium hypochlorite solution (13% free chlorine) with one drop of detergent (Triton X-100, from Serva) for 30 minutes followed by three rinses with sterile, distilled water. For some of the seeds we tried to uncover the embryo but the very brittle endosperm and the fragile nature of the embryo complicated this endeavour. The best results with respect to sterility and germination rate were obtained with removal of only the external layers of the seed coat so the white endosperm was not affected. Half of each batch was sown on a different medium. One medium was full-strength Murashige & Skoog medium (1962) supplemented with 3% sucrose and 0.2% Gelrite® and the other one was Seramis® (Effem, Verden/Aller) soaked with MS medium containing 3% sucrose. The media were adjusted to pH 5.8 and filled in 100ml Erlenmeyer flasks. These were autoclaved for 30 minutes at 120°C and 120kPa.

Figure 7: Stem culture of *T. peltatum in vitro*, Institute of Organic Chemistry, University of Würzburg. (Photo: H. Rischer)

Germination occurred within three months though the overall percentage was very low (10%). Neither the free embryos nor seeds sown on MS media solidified with Gelrite® germinated. The cryptocotylar germination (see above) was clearly observed *in vitro*. When the plantlets were about 5 cm high their growth slowed. We conclude that the plantlets were not sufficiently supplied with nutrients on the Seramis substrate. The stems were therefore cut above the cotyledons, and the apical parts were aseptically transferred to flasks containing one-fifth strength MS media solidified with 0.2% Gelrite® and containing 3% sucrose. On this medium the plants grew exceptionally well when transplanted monthly to fresh medium (Figure 7). If the plants are not transplanted in this way, they will not grow well.

Currently we are trying to obtain callus cultures, *i.e.* completely undifferentiated cells using different organs of the specimens as explants. It is hoped that these cultures will grow faster and produce the typical naphthylisoquinoline alkaloids as well as their biosynthetic precursors.

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